U. S. Patent Application No. 10/697,083

Trends Biomater. Artif. Organs, Vol 18 (2), January 2005 *http://www.sbaoi.org*

Comparison of Artificial Saliva Substitutes

A. Preetha and R. Banerjee
School of Bioscience and Bioengineering
Indian Institute of Technology, Bombay
Powai, Mumbai-400076, India
E-mail: rinti@cc.iitb.ac.in

Abstract:
Human saliva consists of water, glycoproteins, enzymes, antimicrobial substances and electrolytes. From biophysical point of view, saliva is a viscoelastic fluid with distinct surface activity. Commercial artificial saliva used in salivary gland disorders should resemble normal saliva in biophysical properties. In this study we evaluated the biophysical properties of saliva and existing saliva substitutes. We compared three existing substitutes Saliveze, Xialine 1, and Xialine 2. Saliveze is based on carboxymethylcellulose and the others on xanthan gum. The choice of carbohydrate polymers is justifiable because of mucoadhesive nature. Both Saliveze, and xialine 1 are Newtonian fluids (viscosity of 5.71 cP and1.36 cP respectively) where as normal saliva is non- Newtonian (15.51 cP – 2.75 cP at shear rates 0.5 - 94.5 s^{-1}). Similar to normal saliva Xialine 2 is shear-thinning fluid but has a lower viscosity (6.60 - 3.78 cP Vs 15.51 - 2.75 cP at shear rates 0.5 – 94.5 s^{-1}). Shear thinning property is essential to ensure proper flow and cleansing action. The surface activity of both saliva and the substitutes has not been dealt with in the literature. Minimum surface tension of saliveze, xialine 1 and xialine 2 are 64.17, 66.15 and 64.89 mN/m respectively where as that of natural saliva is 24.85 mN/m. Also, unlike natural saliva, none of the substitutes studied showed hysteresis in their surface tension - area isotherms. The low minimum surface tension is essential for saliva film formation on the oral mucosa. The existing substitutes fall short of required biophysical criteria and modifications are required to improve them. We need surface active, shear thinning and mucoadhesive polymer as saliva substitute, possible additions could be surface-active phospholipids, known to be present in saliva and/or mucin, the predominant surface-active protein of natural saliva. Consideration of the biophysical criteria could lead to future development of improved substitutes.

Keywords : Artificial saliva, Surface tension, Viscosity, Newtonian Fluid, Non-Newtonian Fluid

Introduction:

Human saliva is a complex fluid secreted by the major and minor salivary glands and the secretion is under the control of the autonomic nervous system. The three major salivary glands are parotid, sublingual and submandibular. Daily secretion of saliva in human is about 1.5 liters and its normal pH is slightly alkaline (7.4). Saliva contains organic and inorganic substances suspended in an aqueous medium. Besides glycoproteins, like mucin, it contains digestive enzymes like lipase, amylase etc. Other compounds, such as lactoferrins, cystatin, histatin, thiocyanate ion and immunoglobulins are also present (1). Presence of lipids, both neutral and polar, has also been reported (2).

Saliva has several distinct functions namely

cleansing, lubrication, mucosal integrity, buffering, remineralisation, digestion and antimicrobial action. The saliva washes away the food particles from oral mucosa synchronously by the muscle activity, moving debris from teeth and soft tissues progressively towards the back of the mouth and eventually swallowing occurs. Glycoproteins in saliva are responsible for the viscoelastic character of it giving a lubricative film, which enables free movement of oral tissues. The mucin and electrolytes in saliva maintains the oral mucosa in its hydrated state and thus providing mucosal integrity. The most prominent buffering agents in saliva are bicarbonate and phosphate ions and these agents protect the dentition from demineralization. Ions such as phosphate, calcium, and fluoride help for the remineralisation of teeth by promoting surface binding to the hydroxyapetite surface. Enzymes like amylase and lipase play their role in the digestion function. The antimicribial action of saliva is due to the presence of lactoferrins, immunoglobulins, cystatin, histatin and thiocyanate ions. Thus these compounds altogether give saliva a complexity and also distinct rheological and interfacial properties, which accounts for its normal functioning (3).

Salivary gland dysfunction can be due to functional or morphological disorders resulting in qualitative and quantitative changes of saliva. Salivary gland dysfunction is a relatively common problem, which results in the symptoms of a dry, or scalded mouth, difficulties with speech, problems with eating, mucosal infections, denture intolerance, sialadenitis, increased dental caries and periodontal disease (1). The management of patients with salivary gland dysfunction requires enough stimulation of the residual gland function with sialogogues or, in severe cases, use of artificial saliva (3). The present saliva substitutes are intended to act as a replacement of the mucoadhesive, lubricative and protective function of the natural saliva. They are not used as substitutes for the digestive and enzymatic actions. The saliva substitutes must be as close as possible to the natural saliva in terms of composition as well as in biophysical properties. There have been very few studies on biophysical characterization of normal and artificial saliva. In this paper, we have evaluated the biophysical properties of natural as well as some of the existing saliva substitutes.

Materials and Methods:

Materials

All the chemicals used such as Sodium carboxymethylcellulose, Sodium fluoride, Sorbitol, (Loba Chemie, Bombay) Potassium chloride (E. Merck, Bombay), Sodium chloride (SRL, Bombay), Magnesium chloride, Calcium chloride, Di-potassium hydrogen orthophosphate, Potassium di-hydrogen orthophosphate (Glaxo, Bombay), Acetone and Methanol (SRL, Bombay) were of analytical grade. Phosphatidylethanolamine (≥99.9 % pure) was obtained from Sigma (St. Louis, USA). Methyl p-hydroxybenzoate was synthesized in one of our associated labs. Food grade Xanthan gum (Loba Chemie, Bombay) was used. Spirit of lemon was obtained from the lemon fruit. De-ionized water was used for all the experimental purposes.

Methods

Preparation of artificial saliva

Three of the existing saliva substitutes namely xialine 1, xialine 2 and saliveze were prepared in our laboratory. Xialine 1 and xialine 2 is based on xanthan gum and the saliveze is based on carboxymethylcellulose. The composition of these three substitutes was obtained from

the references 3 and 4. Substitutes were prepared using the main constituents of the commercial products. The compositions of the three substitutes are shown in Table 1.

Table 1: Composition of artificial saliva

Artificial saliva → Components ↓	Xialine 1 g/l	Xialine 2 g/l	Saliveze g/l
Xanthan gum	0.92	0.18	N
Sodium carboxymethylcellulose	N	N	10
Potassium chloride	1.2	1.2	0.62
Sodium chloride	0.85	0.85	0.87
Magnesium chloride	0.05	0.05	0.06
Calcium chloride	0.13	0.13	0.17
Di-potassium hydrogen orthophosphate	0.13	0.13	0.80
Potassium di-hydrogen orthophosphate	N	N	0.30
Sodium fluoride	N	N	0.0044
Sorbitol	N	N	29.95
Methyl p-hydroxybenzoate	0.35	0.35	1.00
Spirit of lemon	N	N	5 ml

N – Not present

Collection of natural saliva

Using all aseptic precautions, fresh human saliva was collected from clinically asymptomatic healthy voluntary donors with a 10 ml disposable plastic syringe. All volunteers were examined clinically and any dental and mucosal abnormalities were ruled out. Openings of the salivary ducts were found to be normal. Subjects were asked to rinse the mouth thoroughly with distilled water and to keep the mouth open for 3-5 minutes. The saliva was then collected from under the tongue by keeping the mouth in downward and forward position.

Viscosity measurement

The viscosity of each sample was tested at 37 °C over a wide range of shear rate from 0.512 s-1 to 94.5 s-1 using a rotational co-axial viscometer (Contraves LS 30, Zurich, Switzerland).

Surface tension studies

This was done using a Langmuir Blodgett instrument (KSV instruments Ltd., Finland). This is a Teflon trough, which holds the sub phase (the liquid which supports the monolayer on its surface) and is thermostated by circulating water in channels placed at the

bottom of trough. Thus, the temperature can be controlled from room temperature to 60°C. The surface area of the trough can be varied by sweeping movable barriers (made up of delrin) over the surface of the trough. The surface pressure can be continuously monitored during the compression and expansion movements of the barriers. The surface pressure is the difference between the surface tension of the sub phase in absence and presence of a monolayer on it. The measurement of surface pressure is based on Wilhelmy Plate-method. The Wilhelmy plate is partially immersed into the sub phase and the three different forces acting on this plate are due to gravity, surface tension downwards, and due to buoyancy upwards. The resultant of these three forces (during the partially immerged) is then converted into surface pressure (mN/m or dynes/cm) with the help of the dimensions of the plate.

In our surface activity measurements, we took de-ionized water as the sub phase and temperature was kept at 37 ± 1°C. Teflon coated LB-mini-trough, (Minitrough KSV instruments Ltd., Finland) was used for all the studies. The amount of sample used for each measurement was 100 mg. A Platinum Wilhelmy plate was used to sense the change in surface pressure during the experiment. The trough was cleaned with de-ionized water, methanol, acetone and de-ionized water (again) in sequence several times. Initial surface tension of sub-phase was recorded after dipping the Wilhelmy plate to the sub-phase and zeroed. The entire sub phase surface was cleaned up to zero surface pressure before spreading sample. Using a micro syringe of 50 µl capacity, we spread the sample on the surface of the sub phase as tiny droplets to get a uniform monolayer. The monolayer was compressed and expanded with barrier speed of 120 mm/min, (1 cycle = 1 expansion + 1 compression) without any wait time between compression and expansion. Surface pressure-area isotherms were recorded on KSV, Minitrough LB software (Windows 95/98/NT/ 2000). We obtained a plot of surface pressure as a function of area from this experiment (an isotherm) and the quantities of our interest were calculated from this plot.

Results:

Viscosity

The viscosity of Xialine 1 showed shear thinning behaviour but the range was from 6.38 – 3.78 cP. Xialine 2 and saliveze were Newtonian with a mean viscosity value of 1.36 cP and 5.71 cP respectively. As evident from Fig 1, all the substitutes had viscosities away from the natural saliva viscosity. All the twelve natural saliva samples showed shear thinning behavior. The range of mean viscosity was from 15.5 – 2.8 cP.

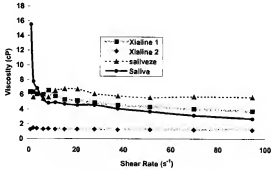

Fig 1: Shear Dependence of Viscosity

The above curve is the mean curve of 12 natural saliva sample data and the black curve the best fitting curve for this fluid.

Surface tension

The surface activity of artificial saliva and natural saliva is evident from the surface tension-area isotherms shown in Fig 2.

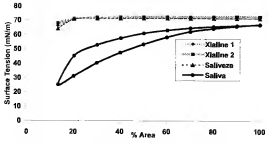

Fig 2: Surface tension – Area isotherms.

The saliva isotherm is the mean of 12 normal human saliva samples and the other three curves are the mean of 3 trials.

In these isotherms we have shown the first compression and first expansion as one cycle. Unlike natural saliva, none of the artificial saliva showed hysteresis in their isotherms. From our experiments we have found out two surface tension terms namely the minimum surface tension & equilibrium surface tension. Minimum surface tension is the surface tension attained by the monolayer of the material at maximum compressed state and the equilibrium surface tension is that at maximum expanded state. These values for saliva substitutes and saliva are shown in Table 2.

Table 2: Surface tension of artificial and natural saliva

Sample	Minimum Surface Tension (mN/m)	Equilibrium Surface Tension (mN/m)
Saliveze	64.17	71.46
Xialine 1	66.15	72.63
Xialine 2	64.89	72.65
Natural Saliva	24.85	67.24

Possible Modifications in Artificial saliva

We also tried some modifications in the artificial saliva. The first thing we studied was the concentration effect. The effect of concentration was done with the xanthan gum in electrolytic solution and the concentrations studied were 0.1, 0.2 and 0.5% by weight. All the three solutions showed non-Newtonian behaviour in viscosity, but the range of values were different. The mean viscosity ranges of 0.1%, 0.2% and 0.5% xanthan gum solutions were 6.37 – 4.92 cP, 82.81 – 20.98 cP and 579.67 – 23.84 cP respectively. The bar graph in Fig 3 shows the viscosity values at selected shears of the 0.1% and 0.2% solutions in comparison with that of natural

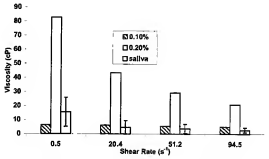

Fig 3: Effect of concentration of xanthan gum on viscosity

All the bars are represented with their standard deviation. Those having no error bars represent zero standard deviation. The bars for saliva are mean of twelve sample values and all other bars are mean of three trial values.

Saliva. In this comparison graph we have not depicted the 0.5% solution data. However, it had extremely high values (the maximum viscosity was 37 times greater than that of natural saliva).

The surface activity was also studied with respect to the concentration change of the xanthan gum. The minimum surface tension of 0.1%, 0.2% and 0.5% solutions were 71.10 mN/m, 58.xy mN/m and 56.nn mN/m respectively. Natural saliva was showing only 24.85. mN/m. The surface tension area isotherms of the above solutions are represented in Fig 4.

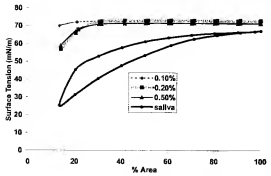

Fig 4: Effect of concentration of xanthan gum on Surface activity

The saliva isotherm is the mean of 12 normal human saliva samples and the other three curves are the mean of 3 trials.

There is literature evidence for the existence of lipids in human saliva (2). So one way to improve the biophysical characteristics of artificial saliva is the addition of lipids to it. We tried to modify the saliveze with the addition of phosphatidylethanolamine (PE), a phospholipid known to present in human saliva. Figure 5 shows the effect of PE addition on viscosity. PE2 addition increases the maximum viscosity of saliveze to 22 fold whereas PE1 addition makes it double. The minimum viscosity value increases from 5.66 cP to 6.73 cP by PE1 addition where as PE2 addition makes four fold increase in this value.

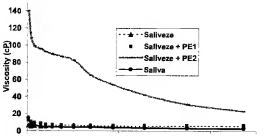

Fig 5: Effect of lipid addition on shear dependence of viscosity

PE1 means concentration of PE as that in natural saliva and PE2 means concentration of PE is ten times greater than that in saliva. The saliva isotherm is the mean of 12 normal human saliva samples and the other three curves are the mean of 3 trials.

The surface tension change on addition of PE1 and PE2 is also interesting and depicted in Fig 6. Here PE2 addition caused promising effect. By the addition of PE2 the Minimum surface tension reduced from 64.17 mN/m to 51.01 mN/m while PE1 addition caused reduction only to 62. 88 mN/m. In the case of the equilibrium surface tension the values for saliveze, PE1 addition and PE2 addition remained more or less the same (71.46mN/m, 71.49 mN/m and 71.95 mN/m respectively). But all the above values were not matching with natural saliva data.

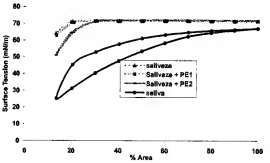

Fig 6: Effect of lipid addition on surface Activity

PE1 means concentration of PE as that in natural saliva and PE2 means concentration of PE is ten times greater than that in saliva. The saliva isotherm is the mean of 12 normal human saliva samples and the other three curves are the mean of 3 trials.

Discussions:

Viscosity

All the three substitutes studied were far away from natural saliva in the case of non-

Newtonian nature. The non-Newtonian character is essential for normal physiological functioning of saliva. Especially for the lubrication of the entire oral mucosa and for the proper mixing with the food materials shear-thinning nature is a must. This nature could also lead to increased flow rate of saliva, which in turn helps for its cleansing action. The very interesting use of shear thinning is that it will allow protection at rest and flow during mastication. Xialine 2 and saliveze were Newtonian and it indicates that these are not efficient in lubrication, cleansing and digestion function. Xialine 1 is having the shear thinning behaviour but the range of value is not matching with natural saliva data. So this also is not up to the mark in viscous character. The viscoelastic nature of natural saliva is mainly due to the mucin content and the shear thinning may be due to the changes in mucin structure leading to a decrease in macromolecular aggregation at high shear and hence a lowering of viscosity with shear. The lowering of viscosity at high and medium shears may lead to thorough mixing of saliva with food and thus may contribute for its proper digestion. From Fig 1 we can see that none of the substitutes have considerable lowering of viscosity with respect to shear change. To sum up the dynamic nature of viscosity of saliva with shear rates help to maintain its normal physiological functions but this nature is not found in any of the substitutes studied and there is a need for modification in viscosity of these substitutes.

Surface Tension

We are using two surface tension terms namely minimum surface tension and equilibrium surface tension. The minimum surface tension corresponds to the tension of the saliva film during the compressive phase of mastication when the muscles of mastication i.e. pterygoids, temporalis, masseter are working. The equilibrium tension can be correlated to the resting condition of the buccal cavity.

The mean minimum surface tension of the normal saliva was found to be 24.85 ± 2.81 (mean + standard deviation) and the mean equilibrium surface tension was 67.35 ± 2.81.The equilibrium surface tension is high indicating the stability of the saliva film in resting condition when the muscles of mastication are not working and jaw is at rest. The minimum surface tension can be correlated to the surface tension of the saliva film when it is completely compressed during mastication and jaw movements. The less value of the minimum surface tension indicates that the saliva keeps the oral mucosa and the tongue moist due to its high surface activity.

The surface activity of the three substitutes studied was not up to the mark when compared with the natural saliva. Figure 5 clearly shows that surface activity of the saliva substitutes is poor compared to that of natural saliva. Also from the table 2 it is clearly evident that the minimum and equilibrium surface tension values for none of the substitutes is matching with that of saliva. Low value of minimum surface tension is needed to ensure the spreading of saliva film on the oral mucosa and hence the proper moistening of the entire oral mucosa. The minimum surface tension of all the substitutes was roughly 2.5 times greater than that of natural saliva. This implies their decreased ability to spread on to the oral mucosa which inturn indicates that they are not giving proper moistening. Similarly the equilibrium surface tension of the substitutes is not exactly similar to that of saliva. All the substitutes have equilibrium surface tension value, which is approximately 5 mN/m greater than natural saliva value. Thus it can be said that unlike the moistening function, the

stability of the saliva film in the resting condition of the mouth is affected to a lesser extent by the artificial saliva. In short all the three substitutes studied are far away from the natural saliva in terms of surface activity.

Possible Modifications in Artificial saliva

We established that all saliva substitutes studied fall short of the biophysical nature of natural saliva. We explored certain modification attempts to alter the properties of the substitutes. The modifications we tried are i) concentration of main constituent and ii) addition of a phospholipid. The effect of concentration was done with the xanthan gum in electrolytic solution. Keeping the electrolytic concentration constant, the gum solutions with weight percentages of 0.1, 0.2 and 0.5 were prepared and the characterization was done. We have chosen these concentrations based on the xialine results. The xialine 2, which was more close to natural saliva, was 0.09% xanthan gum solution so we stared from 0.1% solution. Larsson *et al* reported about the lipids in human saliva. According to them in whole saliva the total amount of lipid was 1.36mg/100ml. Of this 1.36mg, 3.6% is polar lipids. Their data also reveals that phosphatidylethanolamine (PE) is the most abundant phospholipids (9% of the total polar lipids) among the identified polar lipids in saliva (2). Keeping the above fact in mind, we tried to modify the saliveze by adding phosphatidylethanolamine. Two concentrations of PE (PE1 and PE2) were used. PE1 was according to the lipid content of natural saliva (4.4 μg/100 ml of saliva) and PE 2 was ten fold of the PE content in saliva (44 μg/100 ml of saliva).

From our result section 3.3 and figure 3 it is clear that the concentration hike of xanthan gum is possible only up to 0.1%. With 0.2% and0.5% the surface tension was improved to a promising range but the viscosity shoots to 37 fold and 5 fold respectively of the natural saliva data. Thus concentration of xanthan gum allowed is only up to 0.1%, which also is not matching with natural saliva in any of the properties, but there is scope for modification by addition of some other compounds like mucin.

The viscosity of saliveze is shifted towards that of natural saliva with the addition of PE1 where as the surface activity is not much changed by PE1. In the case of PE2 Surface tension change was promising where as the viscosity shooted to very high range (140-23 cP). So addition of PE in the same concentration as that of saliva seems to be reasonable .In short though the addition of PE was promising on the surface activity front, our efforts were only partially successful. Human saliva not only contains polar lipids but also neutral lipids. We only studied the effect of addition of one of the phospholipids (Polar lipids) PE. In fact if we could try the addition of a mixture of lipids (with respect to the natural saliva composition) it will be the best and may give more promising effect.

Another possible modification is the addition of mucin. Mucin is a glycoprotein characterized mainly by its high molecular weight. It has a strong tendency to adsorb on to several interfaces (5). This adsorption leads to a significant protection against bacterial colonization. It is also reported in literature that at physiological pH mucin has minimum surface tension ~ 30 mN/m (5). From our studies it is shown that the minimum surface tension of natural saliva is 24.85 mN/m. So this mucin could be another base material for a promising saliva substitute. There is a need to explore additive combinations of phospholipid enhancers to the mucoadhesive polymers in order to develop more efficient saliva substitutes.

Conclusions:

Biophysical characters of natural saliva has been compared with those of three existing saliva substitutes namely xialine 1, Xialine 2, and saliveze. In terms of viscosity and surface tension these three substitutes were far away from natural saliva. Moreover these quantities are less extensively studied with respect to saliva substitutes and needs to get more attention. Need for modification of existing saliva substitutes is essential to bring them close to natural saliva. We need surface active, shear thinning and mucoadhesive polymer as saliva substitute, possible additions could be surface-active phospholipids, known to be present in saliva and/or mucin, the predominant surface-active protein of natural saliva. Consideration of the biophysical criteria could lead to future development of improved substitutes.

References:

1. S. P. Humphrey, R. T. Williamson, A review of saliva: normal composition, flow, and function. The Journal of Prosthetic Dentistry, Vol. 85, 162 (2001).
2. B. Larsson, G. Olivecrona, T. Ericson, Lipids in human saliva. Archs oral Biology, Vol. 41, 105 (1996).
3. M. M. Ferguson, M. J. Barker, Saliva substitutes in the management of salivary gland dysfunction. Advanced Drug Delivery Reviews, Vol. 13, 151 (1994).
4. A. L. A. Ruissen, W.A. V. Reijden, W. van't Hof , E.C.I. Veerman, A.V.N. Amerongen, Evaluation of xanthan as vehicle for cationic antifungal peptides. Journal of Controlled Release, Vol. 60, 49 (1999).
5. H.Y. Shrivastava, A. Dhathathreyan, B. U. Nair, Molecular organization and aggregation of mucin at air-water interface in the presence of chromium(III) complexes. Chemical Physics Letters, Vol. 367, 49 (2003).